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ORIGINAL ARTICLE

FGF1 protects against APAP-induced hepatotoxicity via suppression of oxidative and endoplasmic reticulum stress

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Summary Acetaminophen (APAP) overdose/abuse is the leading cause of acute liver failure in many countries. Fibroblast growth factor 1 (FGF 1) is a metabolic regulator with several physiological functions. Previous studies suggested that FGF1 promotes differentiation and maturation of liver-derived stem cells. In this study, we investigated the protective effects of FGF1 against APAP-induced hepatotoxicity in mice. APAP markedly increased circulating levels of ALT and AST, while FGF1 significantly inhibited increases in the serum levels of ALT and AST, as compared to littermates. In addition, histopathological evaluation of the livers revealed that FGF1 prevented APAP-induced centrilobular necrosis. Livers exhibited severe inflammation, apoptosis, oxidative stress and endoplasmic reticulum stress in response to APAP toxicity, whereas these changes were reversed by a single injection of FGF1. In conclusion, our findings suggest that FGF1 protects mice from APAP-induced hepatotoxicity through suppression of inflammation, apoptosis, and oxidative and endoplasmic reticulum stress. Therefore, FGF1 may represent a promising therapeutic agent for APAP-induced acute liver injury.

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Introduction

Acetaminophen (APAP), is a safe and popular antipyretic and analgesic drug at therapeutic doses. However, excessive doses of APAP cause acute and severe liver injury [1]. Overdose of APAP causes primary hepatocellular toxicity as a result of production a large number of toxic free radical metabolites such as N-acetyl-p-benzoquinone imine (ANPQI) that depletes glutathione (GSH) and covalently binds to cellular proteins [2]. The depletion of GSH further results in mitochondrial dysfunction, oxidative stress, inflammation, and DNA damage [3—5]. Uzi et al. observed that ER stress and UPR activation were late events in the cascade of responses activated by APAP that coincided with CHOP upregulation [6]. It was further shown that factors involved in innate immunity, including Toll-like receptors (TLRs), dendritic cells, and autophagy contribute to APAP-induced hepatotoxicity [7,8]. Despite extensive studies during the past decade, studies of therapeutic options for APAP-induced liver injury remain rare.

Fibroblast growth factor-1 (FGF-1), also called acidic FGF (aFGF), is a member of the structurally-related FGF family, originally isolated from the brain and pituitary as mitogens [9,10]. Recently, recombinant FGF1 (rFGF1) has been identified as having potent glucose-lowering and insulin-sensitizing effects in the treatment of insulin resistance and type 2 diabetes, without causing hypoglycemia [11]. Furthermore, in various models of nonalcoholic fatty liver disease (NAFLD) mice, rFGF1 effectively improves hepatic inflammation and damage [12]. FGF1 also promotes differentiation and maturation of liver-derived stem cells [13], suggesting its potential therapeutic effects in various liver diseases. Nevertheless, the effect of FGF1 on toxin- or drug-induced liver injury has not been investigated. In the present study, we investigated the protective effect of FGF1 against APAP-induced acute liver damage in mice as well as the potential protective mechanisms.

Materials and methods

Animal experiments and biochemical analysis

Eight-week-old male C57BL/6 mice were obtained from the Animal Center of the Chinese Academy of Sciences in Shanghai, China. Mice were intra-peritoneally injected with APAP dissolved in phosphate-buffered saline (PBS) (Sigma-Aldrich, MO, USA) at doses of 500 mg/kg body weight to induce hepatotoxicity, or with an equal volume of PBS in controls. To investigate the effects of FGF1, 30 minutes after APAP administration, FGF1 solution was injected intraperitoneally at 1.0 mg/kg. Mice were anesthetized to collect blood and were sacrificed to obtain livers after 6 h treatment. All experimental procedures were approved by the ethics committee of Wenzhou Medical University and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

A portion of liver was isolated and fixed in 4% paraformaldehyde and the remaining parts were immediately stored at −80 °C. Serum was obtained by centrifugation at 3000 rpm for 10 min. serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using diagnostic ALT and AST test kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Serum levels of high mobility group box 1 protein (HMGB1) were quantified using sandwich immunoassays from Shino-Test Corporation (Tokyo, Japan).

Hematoxylin-eosin staining and immunohistochemistry

Liver tissues were fixed in 4% paraformaldehyde solution for 24 h and were then mounted in paraffin for histopathological examination by hematoxylin-eosin (HE) staining. The transverse paraffin sections were also incubated in 3% H_2O_2 and 80% carbinol for 30 min and then in blocking solution for 1 h at room temperature. Subsequently, the sections were incubated at 4 °C overnight with the following primary antibodies: PDI (1:300) and ATF-6 (1:200, Santa Cruz Biotech, Santa Cruz, CA, USA). Then the sections were incubated with secondary antibodies (1:2000 dilution) IgG-horseradish peroxidase-conjugated for 2 h at room temperature. The reaction was stopped with 3, 3-diaminobenzidine (DAB). The results were imaged using a Nikon ECLPSE 80i (Nikon, Tokyo, Japan).

Western blot analysis

Liver tissues were homogenized in lysis buffer (RIPA:PMSF:NaF = 100:1:5) and then centrifuged at 12000 × g for 15 min at 4 °C. Total cellular proteins were resolved on SDS-PAGE and transferred to PVDF membranes, and were subsequently probed with primary antibodies against TLR4 (Abcam), Bax (Abcam), Bcl-2 (Abcam), CHOP (Santa Cruz Biotechnology), ATF6 (Abcam), XBP-1 (Abcam), PDI (Abcam), and GAPDH (Cell Signaling Technology) followed by incubation with their corresponding horseradish peroxidase-conjugated secondary antibodies. The protein bands were visualized with enhanced chemiluminescence reagents (GE Healthcare, Uppsala, Sweden) and quantified using NIH ImageJ software.

Determination of hepatic IL-6 and TNF-α concentration

Fifty to 70 mg liver tissue was homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 4 mM Na_3VO_4, 40 Mm NaF, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1% protease inhibitor cocktail). TNFα and IL-6 protein levels were measured using their respective ELISA kits (BD, Sparks, MD, USA) according to the manufacturer's instructions. The values were expressed in pg/mg total protein.

RNA isolation and qRT-PCR analysis

Total RNA from 0.1 g liver sections was isolated with TRIzol according to manufacturer's protocol (Invitrogen, Carlsbad, CA) and qRT-PCR was performed as previously described. The sequences of forward and reverse primers are listed in supplementary material (Sup l). The relative quantities of target transcripts were calculated from duplicate samples after normalization to the housekeeping gene *β-actin*.

TUNEL assay

Formalin-fixed paraffin liver sections were sectioned at 5 μm. The sections were stained for TUNEL with the Apop Tag Peroxidase In Situ Apoptosis Detection Kit (Chemicon, CA, USA) according to the manufacturer's instructions.

Measurements of intracellular T-AOC, GSH-Px, MDA and SOD levels

T-AOC, GSH-Px, MDA and SOD concentrations in liver tissues were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

Statistical analysis

The data were expressed as the mean ± SEM. Statistical calculations were performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, USA). Statistical significance was determined by the Student's *t*-test (for comparison of two experimental conditions). *P*-values < 0.05 were considered statistically significant.

Results

FGF1 meliorated APAP-induced liver injury in mice

To evaluate the effects of FGF1 on APAP-induced acute hepatotoxicity, we performed serological and histological comparison between the APAP and APAP + FGF1 groups at 6 h after administration of APAP. As expected, serum levels of both ALT and AST in APAP group increased significantly at 6 h after APAP injection (Fig. 1A, 1B). However, FGF1 replenishment significantly ameliorated APAP-induced increases in circulating ALT and AST. Histological staining of liver sections revealed that the extent of APAP-induced hepatocyte necrosis in the APAP group was much more severe than that of the control group (Fig. 1D). Likewise, the magnitude of the increase in serum levels of the high mobility group protein B1 (HMGB1), a marker of necrosis, in APAP-treated mice was significantly higher than those of controls (Fig. 1C). Liver necrosis induced by APAP was dramatically reversed with replenishment of FGF1. These results suggested that FGF1 plays a protective role against APAP-induced liver injury.

FGF1 ameliorated APAP-induced inflammation in mice

We next evaluated the expression of inflammatory cytokines following APAP stimulation with or without FGF1. As shown in Fig. 2, APAP administration significantly enhanced expression levels of TLR4 that were markedly attenuated by FGF1 treatment. In addition, expression levels of IL-6 and TNF-α protein were higher in the APAP treatment group. FGF1 replenishment significantly reduced levels the inflammatory response proteins IL-6 and TNF-α induced by APAP (Fig. 2C and 2D). As expected, the same tendency was also observed for IL-6 and TNF-α mRNA levels (Fig. 2E and 2F), suggesting that FGF-1 suppressed APAP-induced inflammation in the liver.

FGF1 inhibited APAP-induced apoptosis in mice

Previous reports suggested that apoptosis plays an important role in liver injury induced by APAP. Therefore, in this study, we performed a TUNEL assay and measured expression levels of related proteins associated with apoptosis. Compared with the control group, the numbers of apoptosis-positive cells (bright green fluorescence) increased significantly after APAP treatment, which were inhibited after replenishment of FGF1 (Fig. A, B). Next, we studied the expression of pro-apoptosis protein Bax and anti-apoptosis protein Bcl-2 in liver tissue. Indeed, we found that Bax protein in the APAP-treated group was strongly up-regulated, while Bcl-2 expression was down-regulated, both of which were significantly reversed by FGF1 ($P < 0.05$, Fig. 3C—D). These results strongly suggested that replenishment of FGF1 plays a protective role against APAP-induced apoptosis in liver.

FGF-1 inhibited APAP-induced oxidative stress in mice

An overdose of APAP is known to induce oxidative damage in mice, thereby contributing to hepatic injury. In the present study, we measured hepatic T-AOC, GSH-Px, MDA, and T-SOD levels in each group. Compared with the control group, the levels of T-AOC, GSH-Px, and T-SOD were significantly lower, and levels of MDA were significantly higher in the APAP group, all of which were reversed by FGF1 replenishment (Fig. 4, $P < 0.05$), suggesting that FGF1 significantly inhibited APAP-induced hepatic oxidative stress.

FGF1 reduced APAP-induced ER stress in liver

To evaluate the role of ER stress in hepatotoxicity induced by APAP, western blot analysis was used to quantify levels of the ER stress-associated proteins ATF6, CHOP, PDI, and XBP-1. These proteins were all up-regulated significantly at 6 h after APAP administration, and were all significantly down-regulated by FGF1 replenishment (Fig. 5A—E). Immunohistochemistry was also used to assess the levels of ATF-6 and PDI. Similar to the results from western blot, the numbers of ATF-6-positive cells and PDI-positive cells were significantly greater after APAP treatment (Fig. 5F). However, pharmacological replenishment of FGF1 resulted in a marked attenuation of hepatic expression of ATF-6 and PDI (Fig. 5F). These results suggested that FGF1 prevents APAP-overdose-induced hepatotoxicity by inhibiting ER stress.

Discussion

APAP overdose-induced hepatic toxicity has become the most frequent cause of acute liver failure and accidental death in many countries. Nevertheless, the therapeutic options for this fatal disease are rather limited. The present study showed that FGF1 exerts a significant protective effect against APAP-induced liver damage in mice via suppression of inflammation, apoptosis, oxidative stress and ER stress. In

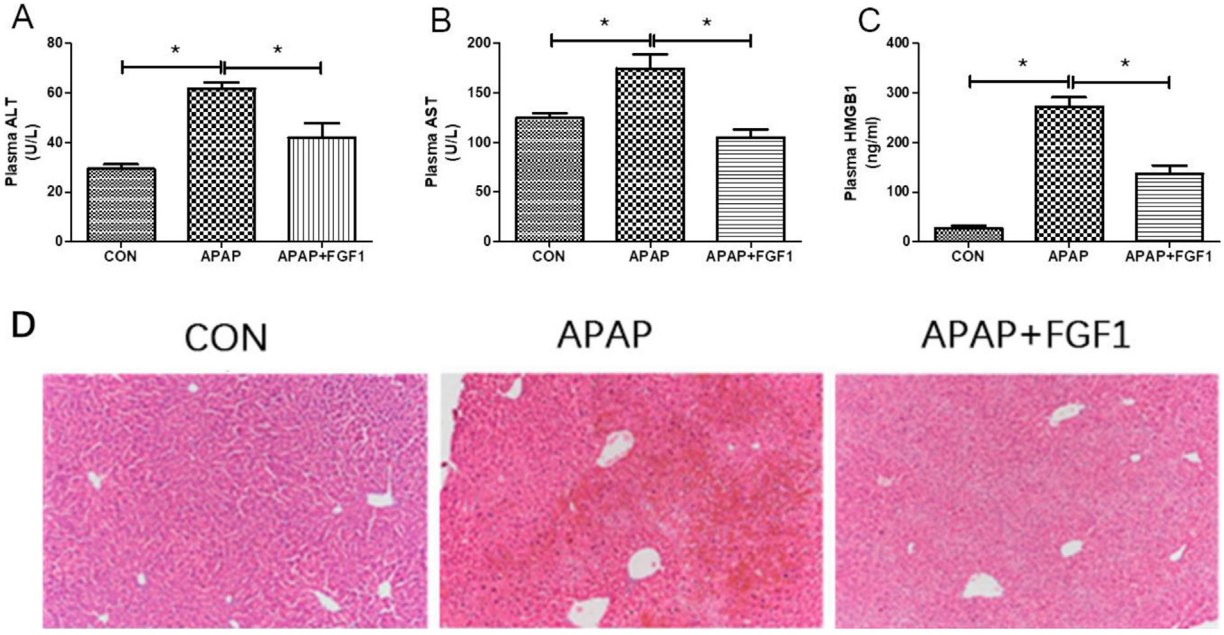

Figure 1 FGF1 reduced APAP-induced liver injury in mice. Serum samples were collected at 6 h after APAP injection for measurement of ALT (A), AST (B) and HMGB1 (C) levels. D. H&E staining analysis of liver sections at 6 h after APAP injection (magnification 40×). Data are expressed as the mean ± SEM, $n = 8$. *$P < 0.05$.

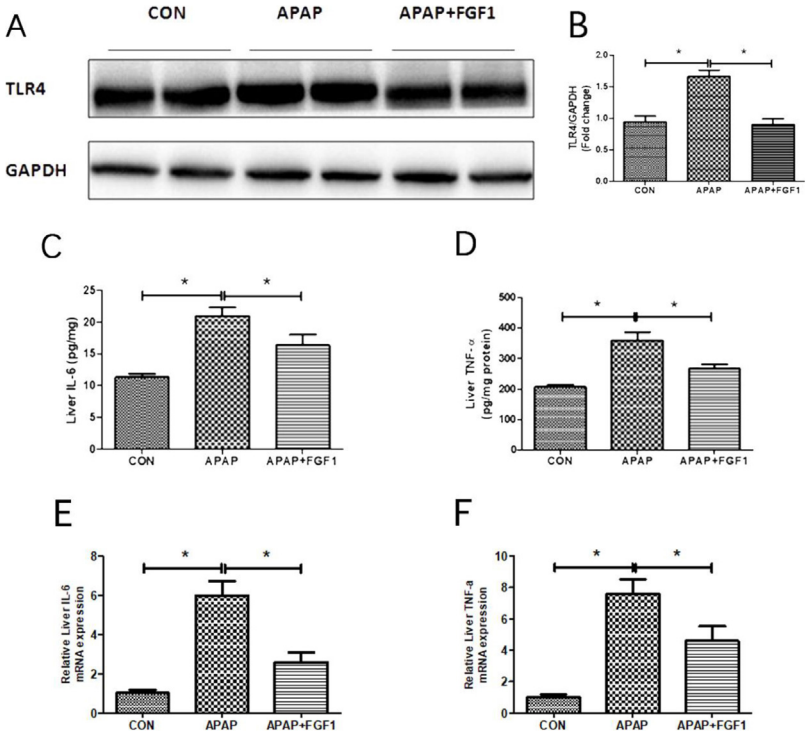

Figure 2 FGF1 decreased the induction of inflammatory cytokines induced by APAP. A. Western blot analysis for TLR4 expression in the liver tissues at 6 h after APAP treatment in mice. GAPDH was used as the loading control and for band density normalization. B. The optical density analysis of TLR4 protein. C-D. Liver IL-6 and TNF-α level were measured by ELISA. Live mRNA expression of IL-6 (E) and TNF-α (F) level was detected. Data are expressed as the mean ± SEM, $n = 8$. *$P < 0.05$.

the current study, mice were treated with FGF1 30 minutes post-APAP administration. We found that FGF1 efficiently attenuated APAP overdose-induced liver toxicity, as shown by lowering serum AST and ALT levels and decreasing hepatocyte injury as shown by H&E staining. These data suggested that a single treatment of FGF1 after APAP administration

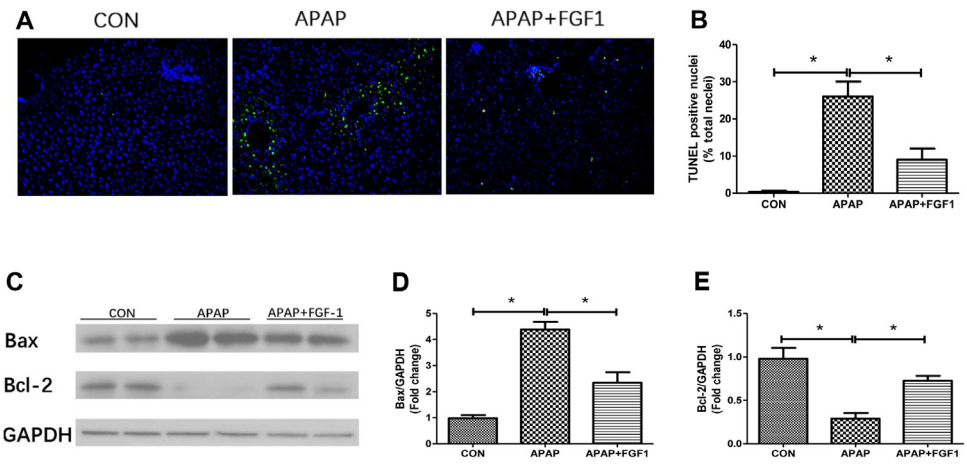

Figure 3 FGF1 inhibited APAP-induced hepatic apoptosis. A. TUNEL fluorescent images. B. Statistical analysis of TUNEL fluorescent images. C. Liver protein levels of Bax and Bcl-2. Statistical analysis of liver protein levels of Bax (D) and Bcl-2 (E).$^{*}P < 0.05$.

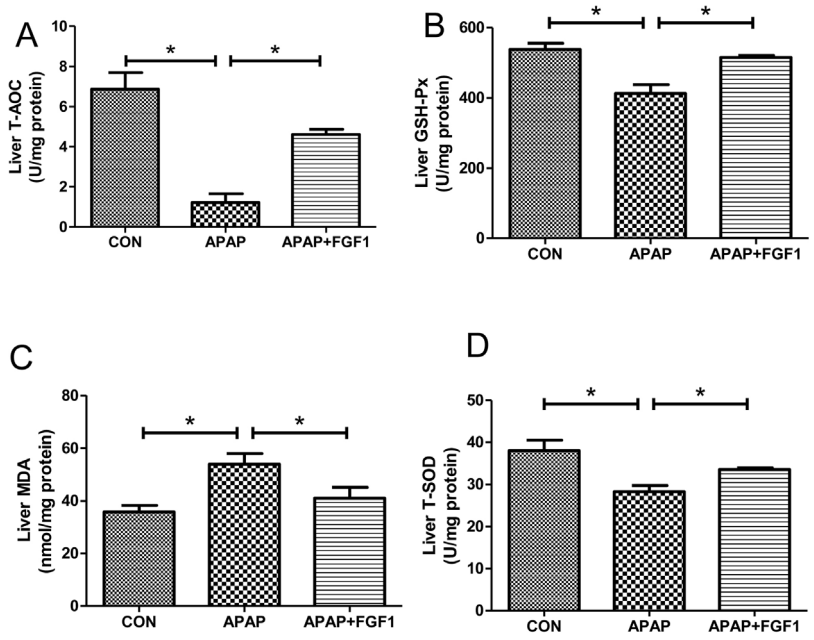

Figure 4 The antioxidant activity of FGF1 in APAP-induced liver injury. A. T-AOC levels, B. GSH-Px levels, C. MDA levels, and D. T-SOD levels were measured by ELISA kits in the liver tissues collected at 6 h after APAP injection. Data are expressed as the mean ± SEM. $n = 8$. $^{*}P < 0.05$.

is a valid therapeutic intervention for APAP overdose-induce hepatotoxicity.

As an autocrine/paracrine regulator, FGF1 has been shown to play a protective role against fatty liver and diabetes-induced injury. Pharmacological administration of recombinant FGF1 (rFGF1) effectively improves hepatic inflammation and damage in leptin-deficient ob/ob mice and in choline-deficient mice, two etiologically different models of NAFLD. Suh et al. observed that long-term treatment of ob/ob mice with FGF1 decreased serum levels of several inflammatory cytokines, including eotaxin, keratinocyte chemoattractant, macrophage inflammatory protein-1β, and interleukin IL-3. Studies reported that FGF1 suppressed oxidative stress and consequently blocked diabetes-induced cardiomyopathy. Our previous study demonstrated that FGF1 treatment ameliorated diabetes-induced nephropathy by inhibiting inflammation via the JNK/NF-kB signaling pathway. In the present study, APAP injection significantly increased serum levels of both ALT and AST and hepatocyte necrosis. FGF1 replenishment significantly ameliorates APAP-induced increase in circulating ALT and AST (Fig. 1A, 1B). Histological staining of liver sections revealed that the liver necrosis induced by APAP was dramatically reversed with replenishment of FGF1 (Fig. 1C). These results suggested that FGF1 plays a protective role in APAP-induced liver injury.

Recent studies on the pathogenesis of APAP-induced acute liver toxicity revealed that not only APAP overdose, but also subsequent inflammatory responses of the immune system critically contribute to the severity of hepatic injury

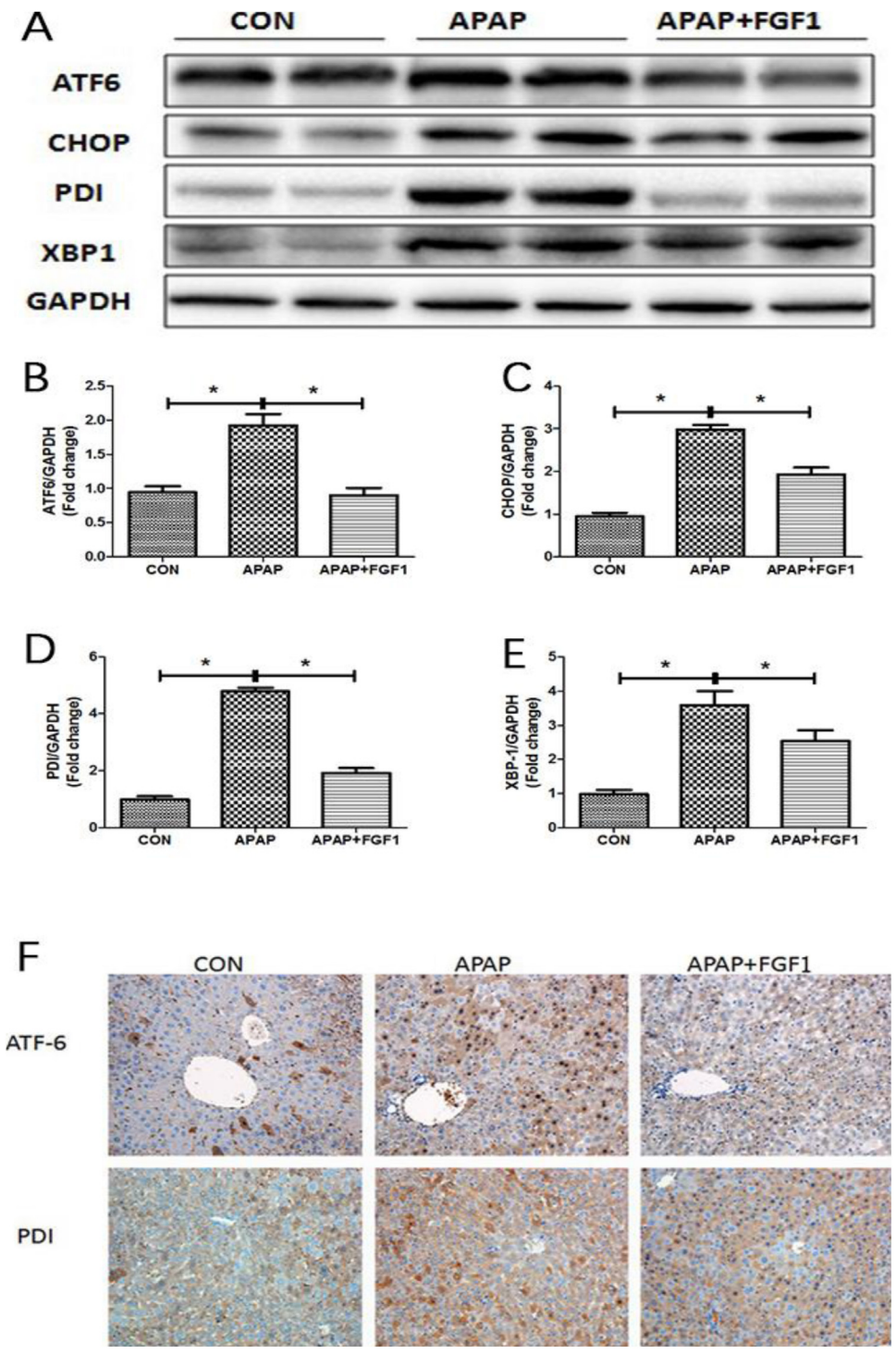

Figure 5 FGF1 replenishment inhibits hepatic ER stress. A. Protein expressions of ATF6, CHOP, PDI, and XBP-1. GAPDH was used as the loading control and for band density normalization. B—E. The optical density analysis of ATF6, CHOP, PDI, and XBP-1 protein. F. Immunohistochemistry for ATF-6 and PDI. Data are expressed as the mean ± SEM. $^{*}P < 0.05$. Magnification was 100×. $n = 8$.

[5,14]. TLR4 plays a pivotal role in the initiation of the immune response after liver injury [15,16] and leads to the production of pro-inflammatory cytokines, including TNF-α and IL-6, for sensitization of hepatocytes to apoptosis [17]. The present study found that APAP administration significantly enhanced the expression of TLR4, IL-6 and TNF-α at the protein and mRNA levels. Furthermore, the numbers of apoptosis-positive cells increased significantly after APAP treatment and protein levels of Bax in the APAP-treated group was strongly elevated, while Bcl-2 levels were down-regulated. As expected, we found that all these changes were significantly reversed by FGF1 treatment, including

markedly reduced expression levels of hepatic TLR4, TNF-α and IL-6 and decreased numbers of apoptosis-positive cells. All these results suggested that FGF1 suppresses APAP-induced inflammation and apoptosis in the liver.

Oxidative stress is a feature of APAP-mediated hepatotoxicity that contributes to the progression of acute drug-induced liver injury by stimulating hepatocellular injury directly [18]. Significant increases in activities of GST and GSH-Px and decreases in MDA concentrations were observed after co-treatment with APAP and pyridoxine in HepG2 cells [19]. FGF1 was found to protect diabetes-mediated oxidative damage through activation of Nrf2 [20]. In this study, APAP treatment significantly decreased levels of T-AOC, GSH-Px, and T-SOD and increased levels of MDA. As expected, FGF1 markedly increased levels of hepatic T-AOC, SOD, GSH-Px and decreased levels of MDA induced by APAP. Therefore, our results suggest that FGF1 effectively protects liver from the APAP toxicity by enhancing enzymatic and non-enzymatic antioxidant defense systems against APAP-induced hepatotoxicity.

ER stress has been suggested to cause hepatic necrosis. ER stress and UPR activation were observed as late events in the cascade of responses to hepatotoxicity, activated by APAP [21]. ATF6 plays a critical role in the pathogenesis of APAP-induced hepatic steatosis via up-regulation of CHOP [22]. Under ER stress, the transcription factor XBP1 is spliced by the endoribonuclease of IRE1, inducing increased expression levels of ER chaperons GRP78 and PDI [23]. Treatment of diabetic mice with non-mitogenic FGF1 (nmFGF1) resulted in significant decreases in BiP and CHOP expression, suggesting that nmFGF-1 independently suppressed ER stress [20]. In a diabetes-induced nephropathy model, FGF1 treatment significantly suppressed diabetes-induced ER stress, as shown by suppressed protein levels of phosphorylated protein kinase RNA like ER kinase (p-PERK), phosphorylated inositol-requiring protein-1α (p-IRE1α), activating transcription factor 6 (ATF6), glucose regulated protein 78 (GRP78) and C/EBP-homologous protein (CHOP) in the kidney, all of which were significantly induced by diabetes. In the present study, ER stress-associated proteins ATF6, CHOP, PDI, and XBP-1 were all up-regulated significantly after APAP administration, suggesting enhanced ER stress. As expected, we found that FGF1 markedly suppressed activation of ATF6, and inhibited expression of ER molecular chaperone GRP78, PDI and the transcription factor CHOP. Therefore, our results suggested that FGF1 effectively protects liver from APAP toxicity by inhibiting APAP-induced ER stress.

In conclusion, our data suggest that FGF1 protects mice from APAP-induced hepatotoxicity through suppression of inflammation, apoptosis, oxidative stress and endoplasmic reticulum stress. FGF1 may represent a promising therapeutic agent for APAP-induced acute liver injury.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

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References

[1] Bunchorntavakul C, Reddy KR. Acetaminophen (APAP or N-Acetyl-p-Aminophenol) and acute liver failure. Clin Liver Dis 2018;22:325—46.
[2] Hodgman MJ, Garrard AR. A review of acetaminophen poisoning. Crit Care Clin 2012;28:499—516.
[3] Gul H, Uysal B, Cakir E, Yaman H, Macit E, Yildirim AO, et al. The protective effects of ozone therapy in a rat model of acetaminophen-induced liver injury. Environ Toxicol Pharmacol 2012;34:81—6.
[4] Li G, Chen JB, Wang C, Xu Z, Nie H, Qin XY, Chen XM, Gong Q. Curcumin protects against acetaminophen-induced apoptosis in hepatic injury. World J Gastroenterology 2013;19:7440—6.
[5] Rivera P, Pastor A, Arrabal S, Decara J, Vargas A, Sánchez-Marín L, et al. Acetaminophen-induced liver injury alters the acyl ethanolamine-based anti-inflammatory signaling system in liver. Frontiers Pharmacol 2017;8.
[6] Uzi D, Barda L, Scaiewicz V, Mills M, Mueller T, Gonzalez-Rodriguez AO, et al. CHOP is a critical regulator of acetaminophen-induced hepatotoxicity. J Hepatol 2013;59:495—503.
[7] Krenkel O, Mossanen JC, Tacke F. Immune mechanisms in acetaminophen-induced acute liver failure. Hepatobiliary Surg Nutr 2014;3:331.
[8] Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev 2012;44:88.
[9] Gospodarowicz D. Purification of a fibroblast growth factor from bovine pituitary. J Biol Chem 1975;250:25152520.
[10] Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biochem 2011;149:121—30.
[11] Suh JM, Jonker JW, Ahmadian M, Goetz R, Lackey D, Osborn O, et al. Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer. Nature 2014;513:436—9.
[12] Liu W, Struik D, Nies VJ, Jurdzinski A, Harkema L, de Bruin A, et al. Effective treatment of steatosis and steatohepatitis by fibroblast growth factor 1 in mouse models of nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A 2016;113:2288—93.
[13] Marsden ER, Hu Z, Fujio K, Nakatsukasa H, Thorgeirsson SS, Evarts RP. Expression of acidic fibroblast growth factor in regenerating liver and during hepatic differentiation. Lab Invest 1992;67(4):427—33.
[14] Antoniades CG, Quaglia A, Taams LS, et al. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. Hepatology 2012;56:735—46.
[15] Pradere JP, Troeger JS, Dapito DH, Mencin AA, Schwabe RF. Toll-like receptor 4 and hepatic fibrogenesis. Semin Liver Dis 2010;30:232—44.
[16] Szabo G, Mandrekar P, Petrasek J, Catalano D. The unfolding web of innate immune dysregulation in alcoholic liver injury. Alcohol Clin Exp Res 2001;35:782—6.
[17] Wu YL, Jiang YZ, Jin XJ, Lian LH, Piao JY, Wan Y, et al. Acanthoic acid, a diterpene in Acanthopanax koreanum, protects acetaminophen-induced hepatic toxicity in mice. Phytomedicine 2010;17:475—9.

[18] Wang X, Wu Q, Liu A, Anadón A, Rodríguez JL, Martínez-Larrañaga MR, et al. Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug Metab Rev 2017;49(4):395—437.
[19] Detoxifying effect of pyridoxine on acetaminophen-induced hepatotoxicity via suppressing oxidative stress injury. Food Chem Toxicol 2018;114:11—22.
[20] Skibba M, et al. Preventive effect of non-mitogenic acidic fibroblast growth factor on diabetes-induced testicular cell death. Reprod Toxicol 2014;49:136—44.
[21] Nagy G, Kardon T, Wunderlich L, et al. Acetaminophen induces ER dependent signaling in mouse liver. Arch Biochem Biophys 2007;459:273—9.
[22] Nagy G, Kardon T, Wunderlich L, Szarka A, Kiss A, Schaff Z, et al. Acetaminophen induces ER dependent signaling in mouse liver. Arch Biochem Biophys 2007;459(2):273—9 [Epub 2006 Dec 3].
[23] Chen L, Li Q, She T, Li H, Yue Y, Gao S, et al. IRE1α-XBP1 signaling pathway, a potential therapeutic target in multiple myeloma. Leuk Res 2016;49:7—12.